Tumor necrosis factor-α and the early vein graft

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Background: **Tumor necrosis factor-α (TNF-α) has been implicated in the blood vessel wall response to hemodynamic forces. We hypothesized that TNF-α activity drives neointimal hyperplasia (NIH) during vein graft arterialization and that anti-TNF-α therapy would inhibit NIH.**

Methods: **Rabbits underwent bilateral vein grafting using jugular vein. All distal branches except the occipital artery were unilaterally ligated to create distinct flow environments between the bilateral grafts. Vein grafts were harvested sequentially up to 28 days for TNF-α messenger RNA (mRNA) quantitation. In separate experiments, animals received short-term or long-term dosing with pegylated soluble TNF-α type I receptor (PEG sTNF-RI) or vehicle. After 14 to 28 days, grafts were analyzed for morphometry, proliferation, apoptosis, and PEG sTNF-RI distribution.**

Results: **Quantitative mRNA assay (TaqMan) revealed shear-dependent ($P < .001$) and time-dependent ($P < .001$) TNF-α expression. TNF-α induction was maximal at day 1 and gradually decreased over time, but was persistently elevated even 4 weeks later ($P < .001$). Low shear (associated with increased NIH) resulted in significantly higher TNF-α mRNA expression ($P = .03$). PEG sTNF-RI was found in high concentrations in the serum and localized to NIH. The high-flow and low-flow vein grafts from treated animals demonstrated similar volumes of NIH compared with controls. PEG-sTNF-RI had only modest impact on vascular wall cell turnover, as reflected by terminal deoxynucleotide transferase-mediated deoxy uridine triphosphate nick-end labeling ($P = .064$) and anti-Ki-67 ($P = .12$) assays.**

Conclusions: **Placement of a vein into the arterial circulation acutely upregulates TNF-α; this expression level correlates with the degree of subsequent NIH. Pharmacologic interruption of this signaling pathway has no significant impact on NIH or wall cellular proliferation/apoptosis, suggesting that early vein graft adaptations can proceed via TNF-α–independent mechanisms. (J Vasc Surg 2007;45:169-76.)**

Clinical Relevance: **Neointimal hyperplasia stands as a fundamental pathology of vein graft failure. This study reveals that low wall shear significantly upregulates early and acute proinflammatory cytokine tumor necrosis factor-α (TNF-α) in vein grafts (with robust neointimal formation), whereas high wall shear is associated with less TNF-α induction and neointimal hyperplasia. Further studies demonstrate that blockage of inflammatory TNF-α signaling fails to abrogate occlusive vein graft lesions. Thus, further research is required to delineate the biologic significance of the TNF-α expression dynamics observed in the early vein graft.**

Autologous vein remains the preferred conduit for surgical bypass constructions. However, approximately 20% of the grafts fail early because of neointimal hyperplasia,[1] a lesion characterized by uncontrolled cell proliferation and extracellular matrix deposition. Although the cellular events and molecular mechanisms responsible for this process have not been fully elucidated, inflammatory mechanisms have been proposed.[2] The recruitment and activation of inflammatory cells results in local accumulation of cytokines, chemokines, growth factors, proteases, and other bioactive substances, which in turn, drive cell differentiation, proliferation and migration, and matrix deposition.[3] These events can lead to clinically advantageous adaptations that remodel the vein graft wall to accommodate the relatively higher pressure and flow burden, but they can also result in unwanted consequences, including luminal narrowing and graft failure.

Proinflammatory cytokines, such as tumor necrosis factor-α (TNF-α) have been associated with neointimal hyperplasia in vein grafts.[4-6] TNF-α is a pleiotropic cytokine that holds many properties implicated in neointimal hyperplasia and wall remodeling. For example, it induces production of inflammatory mediators such as intracellular adhesion molecule-1,[7] monocyte chemotactic protein-1, and growth factors,[8] stimulates smooth muscle cell and fibroblast proliferation and migration,[9] and promotes matrix degradation,[10] and thus facilitates reorganization of vascular structure.

We have demonstrated in vivo that TNF-α drives arterial neointimal hyperplasia induced by low wall shear[11] as well as the vascular reorganization associated with arteriogenesis.[12,13] Genetic interruption of p55-initiated TNF-α signaling has successfully abrogated neointimal hyperplasia in murine vein grafts.[14] The established pharmacologic anti-TNF-α therapies for rheumatoid arthritis[15] and inflammatory bowel disease[16] provide tools to further dissect inflammatory mechanisms and offer the potential for rapid clinical translation.

The purpose of the current study was to define the temporal expression pattern of TNF-α in vein grafts with both high and low wall shear and then use this knowledge

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Competition of interest: none.

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to test pharmacologic anti-TNF-α therapies to abrogate occlusive vein graft adaptations. We hypothesized that (1) TNF-α signaling is temporally defined and drives neointimal hyperplasia during vein graft arterialization, and that (2) appropriately timed pharmacologic anti-TNF-α therapy would localize to the early vein graft wall and inhibit vein graft neointimal hyperplasia. To add to the experimental robustness and explore potential mechanisms, we tested these hypotheses in both high and low wall shear grafts created in the same animal.

METHODS

Rabbit vein graft model, including both high and low wall shear grafts. This study conforms to the American Physiological Society's *Guiding Principles in the Care and Use of Animals,* and the *Guide for the Care and Use of Laboratory Animals* (NIH Publication No. 85-23, revised 1996). Male New Zealand White rabbits (3.0 to 3.5 kg) underwent bilateral jugular vein interposition grafting, followed by immediate unilateral distal carotid artery branch ligation to create two distinct flow environments within the same animal.[17]

Rabbits were premedicated with ketamine (30 mg/kg intramuscularly), intubated, and anesthetized with inhaled isoflurane. Intravenous heparin (1000 units) was administered, and bilateral external jugular veins and common carotid arteries were exposed through a vertical midline cervical incision. Vein bypass grafts were constructed using an anastomotic cuff technique.[17] Briefly, bilateral external jugular veins were harvested (3 cm in length). Polymer cuffs consisting of a 1-mm body loop were fashioned from a 4F endovascular catheter (Terumo Medical Corp, Elkton, Md). Jugular vein ends were passed through a cuff, everted, and fixed using 8-0 silk. The carotid artery lumen was then exposed using a 2-cm arteriotomy, and the cuffed, reversed vein ends were inserted. A second 8-0 silk was used to secure the artery around the cuff. Finally, 1.0 cm of carotid artery back wall between the cuffs was excised to permit vein graft extension. Unilateral reduction in vein graft flow was accomplished by placement of 8-0 silk suture ligatures to completely occlude all branches except the occipital artery (Fig 1).

TNF-α mRNA quantification in the early vein graft. Five animals for each time point were euthanized at 1, 3, 7, 14, and 28 days. Four normal and unmanipulated jugular veins were harvested from separate animals and served as time 0 baseline controls. High-resolution video images were obtained both at graft placement and harvest and used to determine external graft dimensions. Mean blood flow rate was recorded using an ultrasonic flow meter with a 2.0-mm T106 probe (Transonic Systems, Ithaca, NY).

Specimens were frozen immediately in liquid nitrogen or immersed in 10% neutral buffered formalin for histologic analysis. Morphologic analyses were completed using both in vivo external graft diameter and cross-sectional measurements (AxioVision 3.1, Carl Zeiss MicroImaging, Göttingen, Germany) on Masson and van Gieson's elastin stained

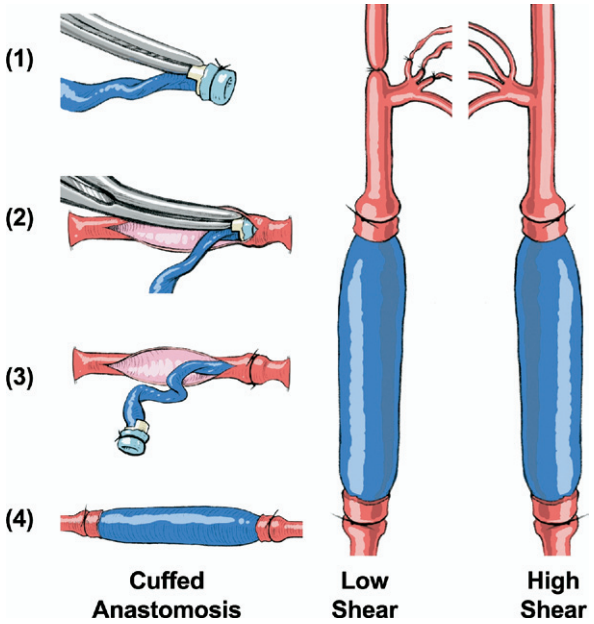

Fig 1. Bilateral vein graft model with differential flow environments. **Left panel,** External jugular vein interposition in the common carotid artery was constructed with cuffed anastomosis. **Right panel,** All distal branches except the occipital artery were ligated to decrease the ipsilateral flow and gain compensatory increase in the blood flow on the opposite side. Adapted from Am J Physiol Heart Circ Physiol 2004;286:H240-5. Used with permission.

specimens, as detailed in our previous report.[17] Briefly, digitized in vivo digital video and fixed microscopic cross-sectional images were collected and analyzed to determine the lumen diameter (D_L) and neointimal cross-sectional area (A_i) for each specimen. Wall shear stress was estimated using Poiseuille's law ($\tau = 32Q\mu/\pi D_L^3$), where Q is the mean flow rate and μ is the viscosity (0.03 poise). Neointimal thickness (T_i) was approximated by [$T_i = 2A_i/(P_L + P_{IEL})$], where P_L is the perimeter of the lumen and P_{IEL} is the internal elastic lamina.

Quantitative real-time two-step polymerase chain reaction (RT-PCR) was performed on paired vein grafts. Total RNA was isolated using TRI and 1-bromo-3-chloropropane (BCP) phase-separation reagents (Molecular Research Center, Cincinnati, Ohio) according to the manufacture's protocol. After treatment with DNase I (Ambion, Austin, TX), reverse transcription was complete using random hexamers (PE Applied Biosystems, Foster City, Calif) to obtain a final complimentary DNA (cDNA) concentration of 20 ng/μL.

RT-PCR for TNF-α (forward primer, AGGAAGAGTCCCCAAACAACCT; reverse primer, GGCCCGAGAAGCTGA TCTG; probe, AGTCAACCCTGTGGCCCAGATGGTC) was performed on a PE 7700 Sequence Detection System (PE Applied Biosystems) by using 200 nM forward primer, 200 nM reverse primer, 50 nM probe, and 10 ng cDNA per 25 mL reaction volume, and TaqMan Universal

Table. Agenda for anti-tumor necrosis factor-α treatments

Experimental groups	*Animals (n)*		*Dosage schedule**		*Harvest time (day)*
	Treatment	*Control*	*Pre-op*	*Post-op*	
Short-term	6	5	0	5	28
Long-term	8	8	1	14	14

*The treatment group received PEG sTNF-RI (10 mg/kg daily) subcutaneously; controls received the same volume of phosphate-buffered saline.

PCR Master Mix (PE Applied Biosystems). RT-PCR was simultaneously run for 18S RNA on all individual samples as an internal control. Samples and controls were assayed in triplicate. The Comparative Ct Method[18] was used for experiments and data analysis. Results are expressed as relative mRNA expression after normalization to 18S ribosomal RNA to minimize variability from sample loading, and finally, calibrated to unmanipulated jugular vein.

TNF inhibition experiments. Pegylated soluble human TNF type I receptor (PEG sTNF-RI; Amgen Inc, Thousand Oaks, Calif), a 20-kd molecule containing a homodimer of human p55 covalently linked to a polyethylene glycol backbone,[19] was used to inhibit soluble TNF-α binding to receptors. Molecular modification of these pegylated receptors, through deletion of 1.4 intracellular domains, serve to reduce immunogenicity while having no impact on ligand binding.[20] Owing to a conserved sequence homology, the compound has been demonstrated to abrogate the adaptive immune response across a range of species, including the rabbit.[19,21] Particularly, Porat et al[21] showed that human p55 is able to recognize and bind to rabbit TNF-α and functionally block rabbit TNF-α bioactivity. In a rabbit septic shock model induced by *Escherichia coli* infusion, recombinant human p55 significantly reduced TNF-α bioactivity in the serum and successfully saved the animals' lives, with a survival rate of 100% for treated group and 55.6% for saline control.[21]

Based on the TNF-α expression results, two anti-TNF-α treatment strategies were used. First, an intense short-term course to block the early, immediate effects of TNF on vein graft adaptation was followed by examination of the morphology at 28 days. And second, a longer-term intervention, designed to explore the impact of prolonged TNF-α inhibition, was initiated preoperatively and continued for the entire 14-day implantation period. Vein grafts were constructed as described earlier, using the high and low shear conditions to examine for potential flow-dependent effects of the inhibitors. Animals were assigned by randomized block design and subcutaneously dosed with either PEG sTNF-RI (10 mg/kg daily) or the same volume of phosphate-buffered saline (PBS) (Table). At harvest, vein grafts were perfusion-fixed in situ with 4% formalin. The middle portion of the graft was processed for morphologic, immunohistochemical, and terminal deoxynucleotide transferase-mediated deoxy uridine triphosphate nick-end labeling (TUNEL) analysis.

Immunohistochemical and TUNEL Analyses. Formalin-fixed sections were rehydrated and treated with antigen retrieval solution (Cat. S2369, Dako, Glostrup, Denmark). Ki-67, a nuclear protein expressed by proliferating cells, was assayed for cell proliferation. Sections were incubated with mouse anti-rat Ki-67[13] (clone MIB-5, 1:400; Dako) at 4°C overnight, then with biotin conjugated goat anti-mouse (Cat M32115, CALTAG Laboratory/Invitrogen, Carlsbad, Calif) at room temperature for 1 hour. Avidin: biotinylated enzyme complex (ABC) and 3,3'-diaminobenzidine (DAB) kits (Vector, Burlingame, Calif) were applied to visualize the specific staining, with hematoxylin counterstain. Anti-PEG sTNF-RI immunostaining was performed in a similar manner to Ki-67, except antigen retrieval and incubation with secondary antibody were omitted. Rabbit anti-PEG sTNF-RI was provided by Amgen and labeled with biotin (Cat 21335, Pierce Biotechnology, Rockford, Ill). Substitution of antibody dilution serum for the primary antibodies served as a negative control. TUNEL assay was performed using an in situ cell death detection kit (Cat 11 684 809 910, Roche Diagnostics, Indianapolis, Ind) and propidium iodide (PI) counterstain. Images were analyzed under confocal. Two individuals blinded to the study counted positively stained cells in two sections for each vein graft.

Confirmation of PEG sTNF-RI localization to the early vein graft. Protein extraction from frozen specimens was performed in 0.05 M Tris (pH 7.5) and 0.2% Triton X-100 using a mortar and pestle. A human sTNF-RI enzyme-linked immunosorbent assay (ELISA) kit (R&D, Minneapolis, MN) was used to determine PEG sTNF-RI content.

Statistical analysis. The effects of time and wall shear were evaluated statistically using two-way repeated measures analysis of variance. Unpaired *t* tests were performed to determine the statistical significance between treatment groups and controls. Results are expressed as the mean ± SEM.

RESULTS

Rabbit model of high and low wall shear vein grafts. Consistent with previous publications using this model,[17] an immediate 11-fold differential in wall shear between high-flow and low-flow grafts was created and maintained during the initial 14 days after implantation (Fig 2, *A*). Secondary to outward remodeling and a compensatory increase in luminal diameter, at 28-days the high shear grafts returned to baseline values. The morphologic impact was an accelerated development of intimal hyperplasia in grafts exposed to the low-flow simulation (Fig 2, *B*).

TNF-α mRNA quantification. RT-PCR for TNF-α revealed shear-dependent ($P < .001$) and time-dependent ($P < .001$) cytokine expression signatures (Fig 2, *C*). TNF-α was induced 142-fold and 94-fold in low and high

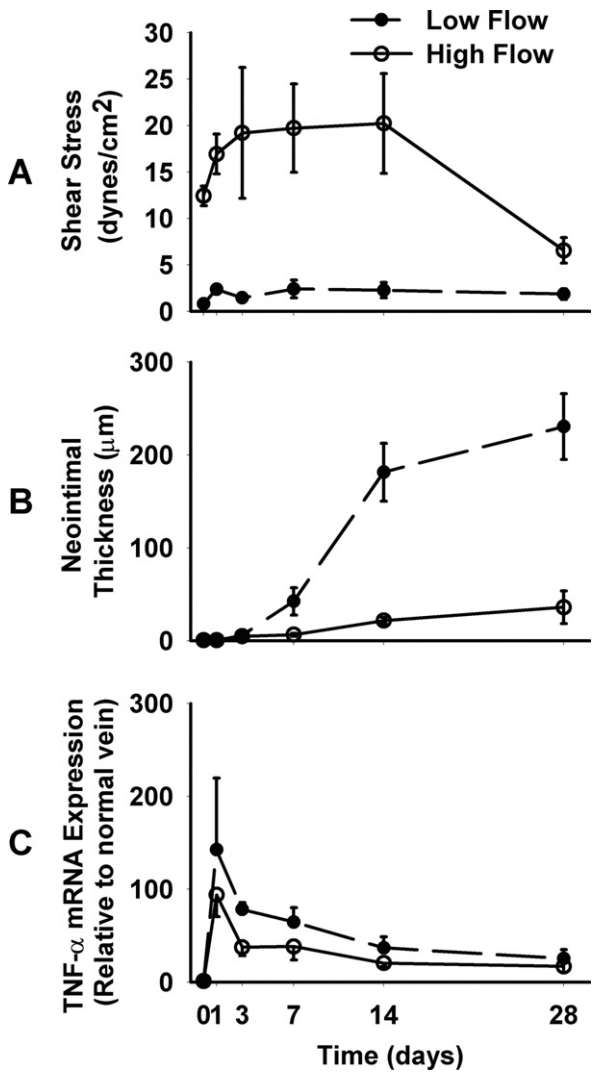

Fig 2. A, Shear stress, **B** development of neointima, and **C** expression of tumor necrosis factor-α *(TNF-α)* messenger RNA *(mRNA)* in low-flow and high-flow vein grafts over time. Both time and flow were identified as independent factors by two-way repeated measures analysis of variance ($P < .001$ for all three end points). Data are presented as means ± SEM.

shear vein grafts, respectively, by the first postarterialization day. This elevation gradually decreased over time but was persistently elevated from baseline with 25-fold and 17-fold induction for low and high shear vein grafts, respectively, at the 28-day time point ($P < .001$). Over the course of the study, low shear (associated with increased wall intimal hyperplasia) resulted in significantly higher TNF-α mRNA ($P = .03$) vein graft wall expression.

Anti-TNF-α intervention. None of the PEG sTNF-RI–treated animals exhibited clinically detectable side effects or complications as measured by body temperature, change in weight, and feeding. However, small superficial skin ulcers associated with the neck incision occurred in two long-term PEG–sTNF-RI–treated animals, and these were present at the time of harvest.

Long-term administration of PEG sTNF-RI resulted in a high accumulation of the compound in the plasma and vascular tissues (97.5 ± 2.3 μg/mL in plasma, 0.52 ± 0.08 pg/μg total protein in inferior vena cava, and 0.08 ± 0.01 pg/μg total protein in carotid artery). Immunohistochemistry revealed that PEG sTNF-RI was largely localized to the cells in developing neointima (Fig 3).

Flow measurements confirmed both treated and vehicle control groups were exposed to similar initial flow conditions (Fig 4). Compared with vehicle administration, short-term or long-term treatment failed to influence neointimal thickness or internal elastic lamina length in either low or high shear grafts (Fig 4). Qualitatively the morphology among all the groups was indistinguishable.

There was a tendency for a higher density of TUNEL ($P = .064$) and a lower density of Ki-67–positive cells ($P = .12$) within the neointima of the group with long-term PEG sTNF-RI treatment compared with controls, whereas an equivalent number of cells positive for TUNEL and Ki-67 were detected within the reminder of the graft wall (media plus adventitia) for both treated and control groups (Fig 5).

DISCUSSION

It has been observed that blood vessels develop occlusive vascular lesions and remodel in response to disordered flow and low wall shear.[22,23] Inflammatory mechanisms drive this blood vessel wall response.[3] Few situations in biology result in a more dramatic perturbation of vessel wall biomechanics than placement of a vein into the arterial circulation; thus, the early vein graft offers an ideal and clinically relevant system to dissect the biology of vascular adaptations to biomechanical forces.

In support of existing paradigms, we demonstrated that low wall shear induced rapid, dramatic TNF-α upregulation in vein grafts that developed robust neointimal hyperplasia, whereas TNF-α was more modestly elevated in high wall shear grafts that formed minimal neointimal hyperplasia.[9,11,24] TNF-α is a pleiotropic proinflammatory cytokine that is able to stimulate the expression of other cytokines, growth factors, and adhesion molecules, thus amplifying the primary signal differentials[25] and leading to the amplified biologic responses.

Consistent with this notion, our results demonstrate that the magnitude of disparity in TNF-α expression is relatively less than that of neointimal hyperplasia. There may not be direct biologic correlations with fold increases in mRNA expression when baseline expression is low and the increases are of such a large magnitude.

Although our immunohistology findings indirectly support a neointimal cellular source for TNF-α, direct TNF-α protein assays have been unsuccessful because of overall low protein accumulation. It is widely accepted that cytokine mRNA levels generally reflect the overall biologic activity of these compounds. The time course demonstrating maximal induction 1 day after implantation closely

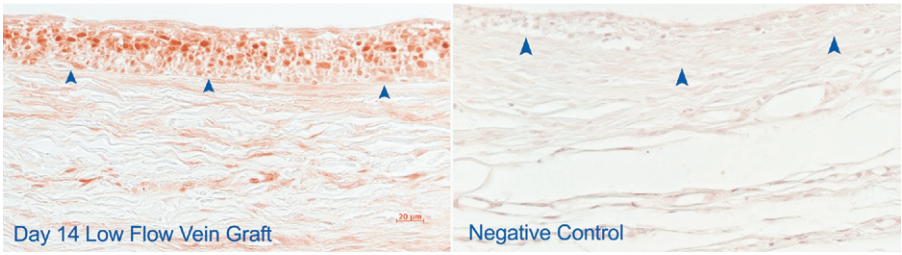

Fig 3. Left panel, Pegylated soluble tumor necrosis factor receptor I (PEG sTNF-RI) immunoreactivity in a long-term treated graft (no counterstain). PEG sTNF-RI colocalizes with cells in the neointima. *Arrowheads* indicate internal elastic lamina. **Right panel,** Negative control (where the antibody was replaced with antibody dilution serum) was completely negative, suggesting the specificity of anti-PEG sTNF-RI immunostaining. Enzymatic 3,3'-diaminobenzidine precipitation was employed to visualize the specific positive staining (original × 400).

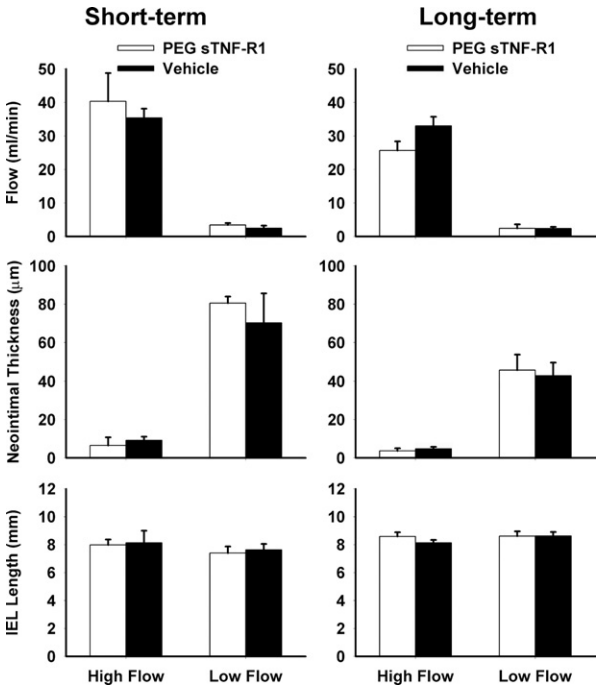

Fig 4. Summary of flow rate at the time of harvest, neointimal thickness, and internal elastic lamina *(IEL)* length for vein grafts with short-term (daily dosing for the first 5 postoperative days of a 28-day observation period) and long-term (daily dosing from 1 day preoperatively until the end of the 14-day observation) anti-tumor necrosis factor-α treatment. No significant difference was detected for both low-flow and high-flow grafts between treated vein grafts and vehicle controls. *PEG sTNF-RI,* Pegylated soluble tumor necrosis factor receptor 1. Data are presented as means ± SEM.

mirrors that of the proinflammatory cytokine interleukin-1β.[6]

The delineation of this early temporal therapeutic "window of opportunity," along with several lines of existing evidence,[14] suggested that systemic anti-TNF therapy could be used to inhibit neointimal hyperplasia in the vein graft. Previous studies in our laboratory demonstrated that less arterial neointima developed in mice deficient in TNF-α when they were exposed to decreased shear, and the reduction was most likely attributed to the soluble TNF ligand.[11] Further studies by Zimmerman et al[26] extended and confirmed these findings. They reported that TNF-α promoted arterial injury–induced neointimal hyperplasia through the type I receptor (p55).[26]

Multiple factors must be considered in light of such high early expression, yet no perceivable biologic effect with TNF-α signaling inhibition. Certainly possible is that neointimal hyperplasia in this specific setting can proceed independent of TNF-α signaling or via compensatory mechanisms. Also, limitations in the rabbit model used in this study may result in a false-negative observation. The cuffed anastomosis we used results in compliance mismatch and does not mimic a sutured anastomosis. Thus, we discarded about 3 mm graft near each cuffed anastomosis. Although we model mid vein graft neointimal hyperplasia, the current study does not explore the biology of anastomotic events directly. The surgery involved with vein graft placement, presence of skin ulcers, and possibly low-grade cerebral ischemia may all result in systemic soluble TNF-α release that may impact the local vein graft wall environment.

Despite high dosages, PEG sTNF-RI binding to the soluble and membrane-bound TNF-α may have been incomplete. However, our dosage (10 mg/kg) was twice as much as the effective dosage (5 mg/kg) for the treatment of rabbit sepsis,[21] and our mean plasma concentration of PEG sTNF-RI was 97 ± 3 μg/mL, which is far above the concentration (5 μg/mL) at which the maximum effects for treatment of rheumatoid arthritis was achieved.[27] As shown in Fig 3, this drug is able to penetrate into the vein graft wall, and the cellular colocalization with the neointima suggests that neointimal cells accumulate TNF-α, which is then recognized and bound by PEG sTNF-RI. Our results do not provide absolute confirmation that the decoy effectively abrogates a biologically significant percentage of TNF-α signaling in rabbits for the vein graft scenario. It is possible that other anti-TNF-α approaches, such as infliximab or etanercept, would offer pharmacologic advantages that would demonstrate clinical benefit.

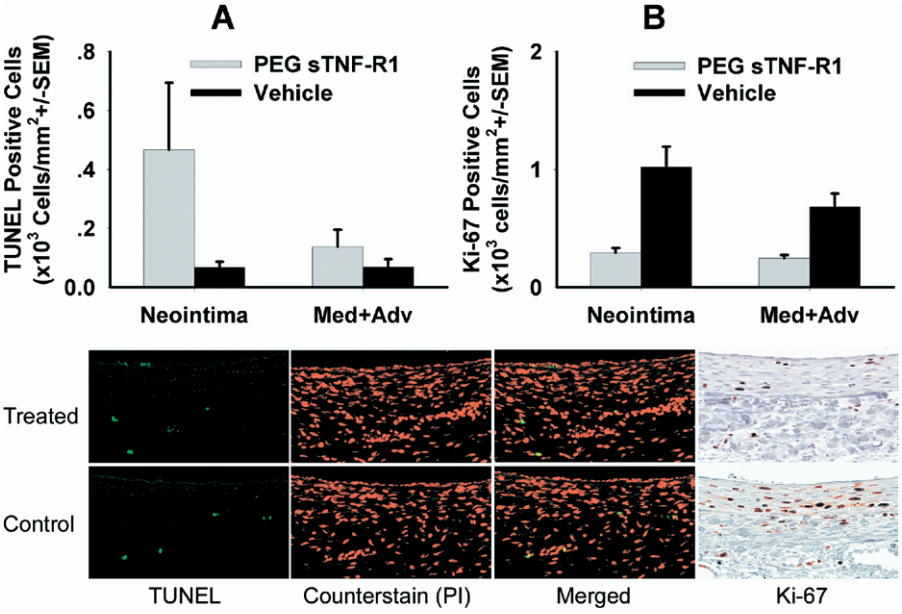

Fig 5. Upper panel, Density of TUNEL **(A)** and Ki-67 **(B)** positive cells in low-flow vein grafts with long-term antitumor necrosis factor-α treatment. Data are presented as means ± SEM. Lower panel, Representative images for TUNEL and Ki-67 from the same animal. The treated vein grafts tended to have more TUNEL-positive and less Ki-67–positive cells in the neointima, but these differences are not statistically significant. *PEG sTNF-R1,* Pegylated soluble tumor necrosis factor receptor 1; *TUNEL,* terminal deoxynucleotide transferase-mediated deoxy uridine triphosphate nick-end labeling.

The short-term dosing regimen opens the possibility that a "catch up" phenomenon may be taking place.[28,29] However, data from the long-term blockage experiment further supports the observation that PEG sTNF-RI did not significantly impact neointimal hyperplasia and remodeling.

Neointimal hyperplasia in the adapting vein graft offers some structural compensation so that the conduit is able to accommodate the pulsatile arterial blood flow under high pressure and wall shear. Although this adaptation facilitates its function in an arterial environment, the neointima may also serve as a fertile soil for the development of other vascular lesions such as atherosclerosis.[30] An effect of anti-TNF therapy could be a shifting of biologic properties of the neointima away from a proatheromatous phenotype. However, neointima in both PEG sTNF-RI–treated and vehicle-treated grafts demonstrated a similar response with respect to cell apoptosis, proliferation, and qualitative structural organization, although long-term end points were not examined.

Although the current findings indirectly conflict with some work from other groups, they probably more realistically point to a sophisticated biology for TNF-α and its pharmacologic blockage in vivo. Zhang et al[14] reported that blocking TNF-α signaling via p55 deletion inhibited neointimal hyperplasia in mouse vein grafts by 40%, and this neointimal attenuation was primarily attributed to graft-intrinsic TNF-α signaling. Their work clearly demonstrated that in the mouse model, TNF-α signaling promotes vein graft neointimal hyperplasia.

In support of this concept is the finding that blocking TNF-α signaling by soluble p75-immunoglobulin G fusion protein (etanercept) resulted in accelerated endothelial cell recovery and reduction of neointimal hyperplasia induced by arterial injury.[31] In our experiment, however, PEG sTNF-RI serves as a decoy to TNF-α ligand; therefore, treatment with PEG sTNF-RI probably substantially decreases (rather than fully abrogates) the binding of TNF-α to the endogenous receptors.

Differential responses to anti-TNF-α therapies in various disease settings have also been noted, although the role TNF-α is considered to be essential in the pathogenesis of the lesions. For example, administration of etanercept, a modified TNF-RII, brought little benefits to patients with Crohn disease,[32] whereas the same dosing regimen led to significant reductions in arthritis activity.[33] Anti-TNF-α therapy can be achieved at different levels of the TNF-α signaling cascade, and several anti-TNF bioactivity reagents have therefore been developed for clinical trials or disease treatment, or both.[34]

Little is known about the impacts of anti-TNF therapy on vein graft neointimal hyperplasia, but a few studies have examined different anti-TNF approaches (soluble receptor[35] and anti-TNF antibody[36]) in rabbit arterial neointimal hyperplasia. The outcomes hold potential controversy, however. Among the confounding factors are the unique receptor kinetics of TNF-α, where incomplete TNF blockage under low TNF-α concentration has been observed owing to the endogenous p75 recruitment of TNF-α and

secondary transfer and activation of downstream signals via p55.[37]

CONCLUSION

Placement of a vein into the arterial system acutely induces TNF-α expression, with an early peak at 24 hours and a lower but sustained expression lasting weeks. This induction is modulated by wall shear stress, with marked augmentation of TNF-α under low shear, which is associated with accelerated neointimal hyperplasia. Based on the PEG sTNF-RI treatment experiments, however, vein graft wall adaptations such as neointimal hyperplasia can proceed independent of TNF-α signaling.

AUTHOR CONTRIBUTIONS

Conception and design: ZJ, AS, BM, DE, MT, SB, CO
Analysis and interpretation: ZJ, AS, BM, DE, MT, SB, CO
Data collection: ZJ, AS, BM, DE, MT
Writing the article: ZJ, SB, CO
Critical revision of the article: ZJ, AS, BM, DE, MT, SB, CO
Final approval of the article: ZJ, AS, BM, DE, MT, SB, CO
Statistical analysis: ZJ, MT, SB, CO
Obtained funding: CO, SB
Overall responsibility: CO

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